Organization of the Peripheral Nervous System: Autonomic and Sensory Ganglia

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Major advances have been made in our understanding of autonomic and sensory transmission and function during the past two decades. These include (i) the establishment of the role of sympathetic and parasympathetic ganglia in relaying neuronal information from the central nervous system to effector organs, (ii) the recognition that enteric ganglia, the third component of the autonomic nervous system, contain independent integrative circuits that control complex local activities, (iii) the evidence for local effector functions of primary sensory nerves in addition to their role in sensory transmission, and (iv) the discovery of plasticity of both autonomic and sensory neurons during disease states and inflammation. A major contribution to these new concepts has been the recognition that in both autonomic and sensory ganglia a variety of transmitters coexist in single neurons. Co-transmission is a widespread phenomenon that enables autonomic and sensory neurons to exert fine and highly regulated control of various functions such as circulation, respiration, digestion, and immune response. This chapter will focus on the general principles and specific features of autonomic and sensory ganglia, with a particular emphasis on their general organization and neurochemical properties. Classical concepts and modern principles of classification of autonomic and sensory ganglia are discussed. ***Key words: peptides/peptide receptors/sympathetic ganglia/parasympathetic ganglia. Journal of Investigative Dermatology Symposium Proceedings 2:1–7, 1997***

Our current understanding of the functional organization of the nervous system derives from neuroanatomical, immunohistochemical, biochemical, molecular biologic, and neurophysiologic approaches that have been instrumental in providing the morphologic and functional substrates for interactions between neurons. Neurons throughout the nervous system are phenotypically heterogeneous in their morphology, projections, electrical properties, and neurochemical composition. A large number of neuroactive substances is expressed in the nervous system. Similarly, there is a variety of plasmalemma molecules involved in cell recognition and adhesion, transmitter reception, and ion channel regulation. The identification of the neurochemical characteristics of individual cells and of the neuronal circuits connecting them is the basis for understanding cell–cell communication, which in turn underlies all nervous system functions. These characteristics apply to both major subdivisions of the nervous system, the central nervous system (CNS), which comprises the brain and spinal cord, and the peripheral nervous system (PNS), which includes peripheral nerves, the autonomic nervous system, and the sensory nervous system. Structurally and functionally, the CNS and PNS are interconnected. Indeed, cell bodies of the peripheral nerves can be located in the brain or spinal cord, or in autonomic or sensory ganglia. Furthermore, the CNS and PNS share a great deal of similarities in their anatomic organization and neurochemistry, even though there are distinct features that are more typical of one or the other component. Clear distinctions and separations among these subdivisions of the nervous system are not always possible, and often there are functional overlaps between different components of the PNS.

This chapter will focus on the PNS and will discuss the general principles and specific features of autonomic and sensory ganglia. The autonomic and sensory ganglia will be considered with regard to their general organization and neurochemical properties. This presentation aims at providing a brief examination of classical concepts of the autonomic and sensory ganglia and a more comprehensive account of recent information that has revealed modern principles of structure and function of autonomic and sensory systems.

THE AUTONOMIC NERVOUS SYSTEM

Autonomic neurons make up an involuntary effector system that regulates the internal environment and maintains body homeostasis (Heimer, 1994). For many years, the control of autonomic functions has been regarded as the result of the antagonistic effects of parasympathetic cholinergic and sympathetic adrenergic transmission, and a major emphasis has been placed on identifying central control mechanisms of autonomic functions. These concepts, however, must now be regarded as an oversimplification, and they do not reflect the complexity of autonomic transmission. Indeed, there have been major breakthroughs in the field of autonomic transmission that have resulted in a profound reconsideration of the mechanisms underlying autonomic functions. First of all, it is now clear that there is a multiplicity of neurotransmitters/modulators in the autonomic nervous system, which include monoamines, pu-

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Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; CGRP, calcitonin gene-related peptide; NPY, neuropeptide Y; SP, substance P; NKA, neurokinin A; NA, noradrenaline/noradrenergic; VIP, vasoactive intestinal polypeptide.

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rines, amino acids, and peptides, in addition to the classical transmitters, acetylcholine and noradrenaline (NA). Second, most neurons contain more than one chemical messenger, which implies the existence of co-transmission or plurichemical transmission (Hokfelt *et al*, 1986; Furness *et al*, 1989; Morris and Gibbins, 1992). Third, a variety of receptor subtypes for autonomic transmitters/modulators has been identified in autonomic neurons, which are also expressed in the CNS and sensory neurons. For instance, as shown in **Fig 1**, an opioid receptor subtype, the μ opioid receptor, which mediates many of the effects of opioids and alkaloids (potent therapeutic drugs commonly used for pain control) and is widely distributed throughout the nervous system, is expressed by autonomic neurons of the intestine (Ji *et al*, 1995; Mansour *et al*, 1995; Sternini *et al*, 1995a, 1996). The cloning and sequences of peptide receptors with the availability of specific antibodies are now providing new research opportunities for the understanding of the mechanisms and the sites of action of transmitters. Fourth, there are autonomic ganglia (the most obvious example being those of the gut) that contain intrinsic integrative circuits that can initiate, sustain, and modulate local activities independent of the CNS (Furness and Costa, 1987). These discoveries indicate a complexity previously unexpected in terms of transmitters/modulators involved in the control of many organ functions, and they point to the importance of peripheral mechanisms *versus* central mechanisms.

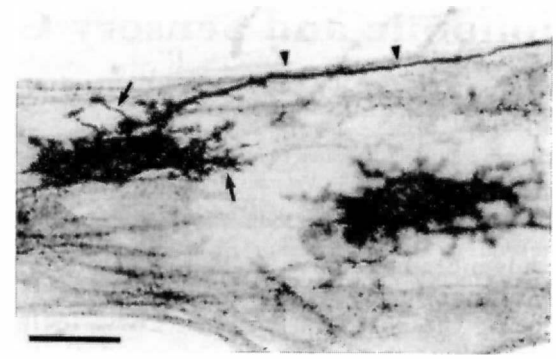

Figure 1. Enteric neurons expressing the μ opioid receptor. Myenteric ganglion of the guinea pig intestine; whole-mount preparation processed for immunohistochemistry using the avidin biotin peroxidase–antiperoxidase method (Sternini *et al*, 1987; Sternini and Anderson, 1992; Sternini *et al*, 1996). Note that these neurons have an ovoidal cell body, with thick dendrites (→ indicate some examples) and a long axonal process (▼). Myenteric neurons with this morphology comprise motor neurons that control smooth muscle contraction, and perhaps interneurons that transmit information between neurons. μ-opioid receptor immunoreactivity was visualized using an affinity-purified antibody raised to the COOH-terminal region of rat μ-opioid receptor; the specificity of this antibody was demonstrated by using cells transfected with opioid receptor cDNAs and by immunoblocking experiments in tissue (Sternini *et al*, 1996). Note that immunoreactivity is mostly confined to the cell surface. *Scale bar*, 50 μm.

Functional Organization of the Autonomic Nervous System The autonomic nervous system comprises three anatomic subdivisions: sympathetic, parasympathetic, and enteric (Langley, 1921). The sympathetic and parasympathetic motor pathways consist of two neurons in series, a pre-ganglionic neuron with its cell body in the CNS and a post-ganglionic neuron with its cell body in the periphery. The latter neuron receives inputs from the pre-ganglionic neuron and innervates target tissues. The enteric subdivision, however, fails to conform to this two-neuron efferent chain description. Enteric neurons are organized into two ganglionated plexuses or ganglia: the myenteric plexus, which is located between the longitudinal and circular smooth muscle, and the submucosal plexus, which is located within the submucosa (Furness and Costa, 1987; Sternini, 1988; Costa and Brookes, 1994; Gershon *et al*, 1994). Myenteric and submucosal ganglia are similar in their organization, in that they contain numerous neurons and supporting cells, which are called "enteric" glia in view of their similarity with the glia cells of the brain. The myenteric ganglia are more compact and larger than the submucosal, thus containing a greater number of enteric neurons (**Fig 2*A*,*B***). The two-neuron chain description is not always applicable to the sympathetic and parasympathetic outflows either. In fact, there are post-ganglionic sympathetic fibers that end in enteric ganglia, which would imply the existence of a third neuron in this chain. Furthermore, pre-ganglionic parasympathetic fibers synapse directly on enteric neurons or project to the gut wall without the participation of post-ganglionic neurons. This has led to the proposition that intrinsic ganglia of the gut are parasympathetic post-ganglionic ganglia on which pre-ganglionic fibers synapse, and they, in turn, send information to be transmitted to the effector cells. This hypothesis, however, is no longer tenable and must be abandoned. The enteric nervous system, as it has been recognized for many years (Kosterlitz, 1968), is able to sustain local reflex activity independently from the CNS, and it contains a much greater number of neurons than the number of efferent fibers running in the vagus nerve (Furness and Costa, 1987), the principal parasympathetic nerve. It is difficult to conceptualize how a divergent ratio of one pre-ganglionic fiber to more than 50,000 supposedly post-ganglionic neurons could occur and could accomplish the coordinated control of digestive functions. In view of the organization of the enteric nervous system and of its relationship with the sympathetic and parasympathetic outflows, it can be stated that the two-neuron chain description of the autonomic nervous system still holds in part for its sympathetic and parasympathetic components; however, it must be kept in mind that (i) the enteric component does not follow this description, (ii) enteric ganglia that receive post-ganglionic sympathetic endings are to be considered the effectors for this set of sympathetic neurons, and (iii) parasympathetic pre-ganglionic neurons that end in the enteric nervous system may synapse directly on enteric neurons.

In many respects, the enteric nervous system shares more homologies with the CNS than any other components of the PNS. It comprises a large number of heterogeneous neurons that can be distinguished on the basis of their morphology, projections, neurochemistry, electrophysiologic properties, and functions. From a morphologic aspect, enteric neurons can be bipolar, pseudounipolar, and multipolar. Broadly speaking, enteric neurons include sensory neurons, interneurons, and motor neurons. **Figure 1** shows a typical multipolar neuron, with many dendrites and a long axonal process, which is likely to be a motor neuron or perhaps an interneuron. The enteric nervous system contains entire reflex pathways that control many digestive functions such as muscle contraction and relaxation, secretion and absorption, and mucosal blood flow (Furness and Costa, 1987; Wood, 1994). That is, sensory information generated by local receptors is processed in local enteric circuits generating outflow that controls motor neurons and influences gut activity either directly or via a chain of interneurons. Motor neurons, which include excitatory and inhibitory neurons to the muscle, secretomotor neurons, and vasomotor neurons, are the final effectors of the neuronal circuits in the enteric nervous system. Furthermore, some enteric neurons project from the intestine to pre-vertebral sympathetic ganglia (Kreulen and Szurszewski, 1979; Furness and Costa, 1987). The enteric nervous system comprises a dense synaptic neuropil and displays excitatory postsynaptic potentials, inhibitory postsynaptic potentials, and inhibitory and excitatory neuromodulation similarly to the CNS. All of this evidence supports the hypothesis that the enteric nervous system performs integrative functions independent of the CNS (Kreulen and Szurszewski, 1979; Furness and Costa, 1987; Gershon *et al*, 1994; Wood, 1994), unlike sympathetic and parasympathetic ganglia that function mainly as relay-distribution centers for information transmitted from the CNS to the effector organs. Therefore, the current concept is that local integrative circuits of the enteric nervous system are organized for functional operations independent

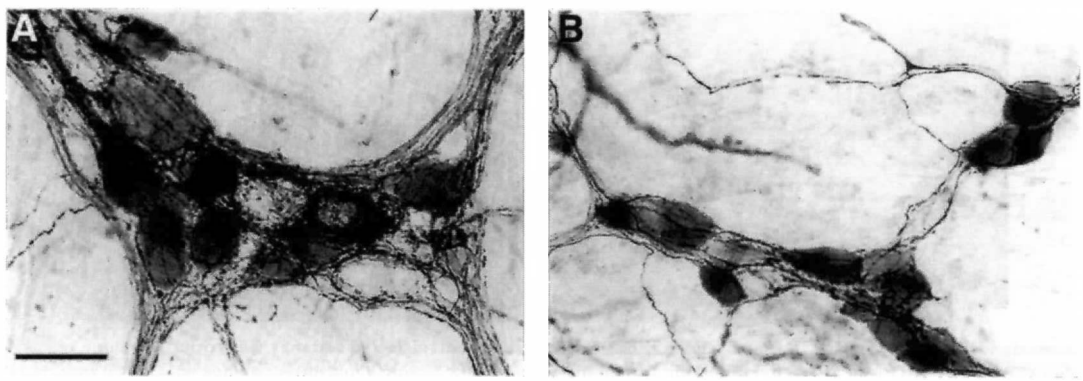

Figure 2. CGRP immunoreactivity in enteric neurons of the myenteric *(A)* and submucosal *(B)* ganglia of rat intestine. Whole mount preparations processed for immunohistochemistry using the avidin biotin peroxidase–anti-peroxidase method as previously described (Sternini *et al*, 1987; Sternini and Anderson, 1992). CGRP immunostaining was obtained with a rabbit polyclonal antibody that recognizes both α- and β-CGRPs and was used at 1:5000; this antibody has been extensively characterized (Sternini *et al*, 1987; Sternini and Anderson, 1992). Note that the myenteric ganglion or plexus (which is located within the smooth muscle, between the longitudinal and circular layers) is bigger and more compact and contains more neurons compared to the submucosal ganglion or plexus (which is located within the submucosa). Also note the dense neuropil in these ganglia, particularly evident in the myenteric ganglion. *Scale bar*, 25 μm.

of pre-ganglionic parasympathetic or other CNS input. The "hard-wired" programs of the enteric nervous system receive inputs from the CNS by way of vagal efferent fibers, explaining the potent influence of a small number of vagal efferent fibers on motility and other effectors over a broad region of the gut.

Neurochemical Properties of Autonomic Neurons and Functional Implications Transmission from autonomic neurons to their peripheral effectors is mediated by a variety of chemical messengers, including the classical transmitters, NA and acetylcholine, and biologically active peptides (Schultzberg, 1983; Schultzberg and Lindh, 1988). Typically, two or more substances are expressed in the same neuron, implying that a single autonomic neuron may affect the activity of its target tissues through the action of multiple substances that contribute to different aspects of neuronal transmission. Indeed, transmitters with apparently opposing effects on effector cells can be found together and presumably are released by the same neuron. The majority of post-ganglionic sympathetic neurons are noradrenergic (NA), and most of these cells contain one of several peptides. A typical example is neuropeptide Y (NPY), the neuronal component of the pancreatic polypeptide family, which is expressed in a large population of NA sympathetic neurons (up to 90% in some ganglia) (McLachlan and Llewellyn-Smith, 1986). Another example includes somatostatin, which is found in NA sympathetic neurons that do not contain NPY (Lindh *et al*, 1986). Indeed, NA ganglion cells can be distinguished on the basis of co-existing peptides (Lundberg *et al*, 1982). NA/NPY neurons of para- and pre-vertebral ganglia innervate blood vessels and the heart, thus playing a role in blood flow regulation (Lundberg and Hokfelt, 1986). NA/somatostatin neurons of the celiac-superior mesenteric ganglion complex project mostly to the submucosal ganglia and mucosa of the intestine and therefore are likely to be involved in mucosal functions, whereas the NA neurons, which contain neither NPY nor somatostatin, project to the myenteric ganglia and are presumably involved in control of gastrointestinal motility (Furness *et al*, 1983; Costa and Furness, 1984). Peptides are also associated with nonadrenergic, cholinergic sympathetic neurons. A typical example is vasoactive intestinal polypeptide (VIP), which is expressed in post-ganglionic cholinergic sympathetic ganglion cells innervating sweat glands and pre-capillary resistance vessels in skeletal muscle, implicating VIP as the substance responsible for the atropine-resistant vasodilatatory effects of these neurons (Lundberg *et al*, 1979, 1981). Calcitonin gene-related peptide (CGRP), a potent vasodilator (Brain *et al*, 1985), and a major sensory marker (Gibbins *et al*, 1987; Ju *et al*, 1987; Kruger *et al*, 1989), has also been found in cholinergic/VIPergic paravertebral sympathetic neurons (Landis and Fredieu, 1986; Lindh *et al*, 1987). Parasympathetic neurons, which have traditionally been considered to be cholinergic, also contain other transmitters or putative transmitter substances. For instance, there are catecholaminergic neurons in cranial parasympathetic ganglia (Teitelman *et al*, 1985). Several peptides, such as VIP, NPY, and CGRP, are also expressed in parasympathetic neurons (Lundberg *et al*, 1980; Landis and Fredieu, 1986; Leblanc *et al*, 1987; Lindh *et al*, 1987).

The enteric nervous system has provided a very useful example of plurichemical expression and transmission. A multiplicity of peptides has been associated with enteric neurons, including those found in sympathetic and parasympathetic ganglia and other peptides. CGRP is a typical example of a putative transmitter with significant expression in the nervous system. It is found in the autonomic nervous system, where it is localized to enteric ganglia **(Fig 2)**, to parasympathetic ganglia (e.g., the otic ganglion and other cranial ganglia) (Silverman and Kruger, 1989); to paravertebral sympathetic ganglia, often co-localizing with VIP (Landis and Fredieu, 1986; Lindh *et al*, 1987), as well as to cranial and spinal sensory neurons (see below and **Fig 3*A***) and to the CNS (Rosenfeld *et al*, 1983). In the enteric nervous system, as many as seven different peptides have been found together with acetylcholine in the same neuron (Furness *et al*, 1992). For instance, cholinergic secretomotor neurons of the intestine in the guinea pig also contain cholecystokinin, CGRP, and dynorphin, and some of them might also contain galanin, neuromedin U, NPY, and somatostatin. Other neurons contain peptides and nitric oxide, a gas that has been recently demonstrated to be a transmitter for inhibitory motor neurons to the smooth muscle of the gut. Nitric oxide is often co-expressed with VIP (De Giorgio *et al*, 1994; Furness *et al*, 1994), but it also co-localizes with dynorphin, galanin, and NPY in inhibitory motor neurons with short projections to the muscle (Costa *et al*, 1986; Furness and Costa, 1987; Furness *et al*, 1988, 1989, 1992). Another example of co-expression of a classical transmitter and several peptides is represented by excitatory motor neurons with long ascending projections to the intestinal muscle, which contain substance P (SP) and related tachykinins, dynorphin and enkephalin, in addition to acetylcholine (Furness *et al*, 1984). Furthermore, the tachykinins include different peptides, such as neurokinin A (NKA), and the extended forms of NKA, neuropeptide κ and γ, in addition to SP (Furness *et al*, 1989; Sternini *et al*, 1989; Furness *et al*, 1992, 1995b). In particular, studies in the enteric nervous system have revealed the multiplicity of effects that peptides have on transmission (Costa *et al*, 1986; Furness and Costa, 1987; Furness *et al*, 1989, 1992; Costa and Brookes, 1994; Furness *et al*, 1995b). Peptides can act as primary transmitters by directly

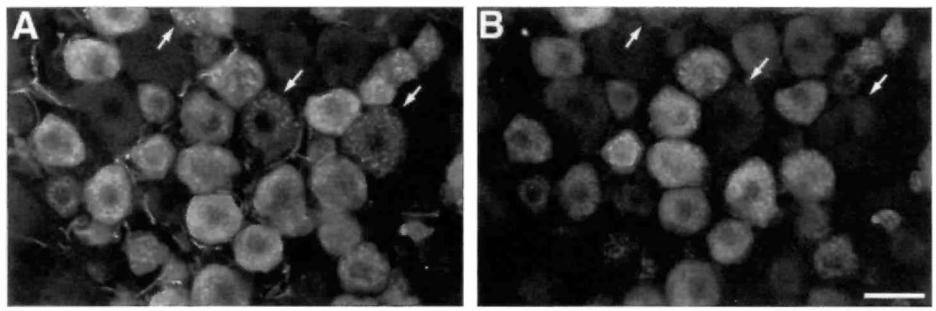

Figure 3. Simultaneous visualization of CGRP and SP/tachykinin immunoreactivities in sensory neurons. The same tissue section of rat dorsal root ganglia at the thoracic level. CGRP and SP/tachykinin immunostaining were obtained using a mixture of guinea pig CGRP antiserum (1:500) and rabbit NKA antiserum (1:500) with double label immunofluorescence. The antibody–antigen complexes were then visualized with secondary antibodies conjugated with different fluorophores (i.e., fluorescein and rhodamine). The CGRP antiserum recognizes both α- and β-CGRPs; the NKA antiserum cross-reacts with the other members of the tachykinin family, including SP. Both antisera have been extensively characterized for immunohistochemistry and have been shown to be specific (Sternini and Anderson, 1992). Note that the co-localization of CGRP and SP/tachykinin immunoreactivities in primary sensory neurons is extensive and that SP/tachykinin-containing neurons represent a subpopulation of the CGRP-containing neurons. → indicate examples of CGRP positive neurons lacking TK immunoreactivity. *Scale bar*, 50 μm.

exciting target cells; as co-transmitters by contributing to the excitability of the effector; as modulators by influencing the excitability of the target cell; or as growth factors by influencing the metabolic state of a target cell. Other substances associated with autonomic neurons include purines, ATP, and γ-aminobutyric acid, the major inhibitory transmitter in the CNS, all of which are likely to influence target cell function (Furness and Costa, 1987; Furness *et al*, 1988; Costa and Brookes, 1994; Peter *et al*, 1995; De Giorgio *et al*, 1996). An obvious question that comes to mind at this point is why do autonomic neurons need so many different chemical messengers to function. The most parsimonious explanation is that co-transmission might be a mechanism by which the autonomic nervous system operates in an efficient and precise manner to respond to multiple functional requirements.

SENSORY NERVOUS SYSTEM

Classically, primary afferent neurons have been defined as neurons with their cell bodies located in spinal and cranial ganglia, which have a peripheral process that conveys information from target organs and a central process that connects synaptically to sensory transmission neurons in the CNS (Kruger, 1987; Dalsgaard, 1988). According to this classical view, sensory neurons function as a receptive, afferent system that activates effector organs in response to stimulation, allowing the maintenance of homeostasis by reacting to internal and external changes. Nerve endings of sensory neurons, also referred to as sensory receptors, transduce mechanical or chemical activity into electrical signals that are conveyed to the CNS via the sensory nerves. Sensory endings may be receptors themselves or be connected with special sensory structures such as the encapsulated endings that include Pacini, Ruffini, and Meissner's corpuscles and Krause's organs. These subserve different aspects of sensations. Sensory endings that do not display morphologic specializations have been referred to as "free-endings." These include the large class of nociceptors that respond to noxious stimuli and often are polymodal, being activated by different types of stimuli whose excitation is associated with the sensation of pain. The lack of specialization in these afferent terminals is inferred by the absence of corpuscular structures and by the lack of synaptic-like contacts (Gottschaldt, 1985). Most of the earlier evidence for sensory function of primary sensory neurons derives from studying somatic afferent neurons, including cutaneous nerves. In more recent years, increasing emphasis has been placed on visceral afferents, the principal role of which is to maintain homeostasis of internal environment by controlling various functions, such as circulation, temperature, digestion, and respiration. Different principles underlie somatic *versus* visceral sensation. Unlike somatic cutaneous afferents, the principal sensation associated with visceral afferents is pain, which is often described as a vague and poorly defined sense of malaise that rarely reaches the level of consciousness and is referred to somatic structures such as the skin and muscle (e.g., visceral referred pain). Furthermore, in the skin, there is a correlation between the discriminative capacity of sensory nerves and the density of innervation, which is lacking in the viscera. Indeed, exploratory organs such a the perioral region of the face and the limbs are supplied by a high density of large, myelinated axons functioning as mechanoreceptors for fine tactile and spatial discrimination (Kruger, 1987, 1988, 1993). Sensory innervation of the viscera is dominated by unmyelinated and slowly conducting fibers, which typically respond to stimuli beyond the physiologic range. The concept that sensory neurons only serve a sensory role has been challenged since the early discoveries of cutaneous vasodilatation following antidromic stimulation of dorsal roots and of transmitter release from primary sensory neurons in the periphery (Hinsey and Gasser, 1930; Dale, 1935). Since then, a large body of evidence has accumulated to support local effector roles of sensory nerve endings in regulating blood flow, vascular permeability, and trophic and immunologic processes as well as autonomic and visceral activities.

Primary sensory fibers have been typically classified in three groups based on their diameter and conduction velocity, which have been correlated to different functions. For instance, C fibers are thin unmyelinated fibers consisting of polymodal nociceptors and a smaller portion of mechanoreceptors. Similarly, the small A fibers carry nociceptive information, but they also carry non-nociceptive information, whereas the large, myelinated A fibers carry non-nociceptive information from the muscle and joints. A convenient classification of afferent neurons is based on the size of their cell bodies, with the small cells mostly, but not always, giving rise to small diameter, thin axons, and the large cells mostly emitting the larger diameter, myelinated axons. Even though there are reservations about how clear cut a separation there is between these two groups of cells, including the fact that there are intermediate sized cells, on functional grounds this dichotomy stills holds quite well. More recently, however, new approaches have been proposed for the classification of sensory neurons, which take into account the chemical properties of the neurons. For instance, it has been demonstrated that functionally different types of primary sensory neurons contain different types of carbohydrate differentiation antigens (Dodd *et al*, 1984a, 1984b), and it appears that there is a correlation between functions and chemical messengers expressed in sensory neurons (Jessell and Dodd, 1986).

Finally, it is important to mention that there is now compelling evidence for the presence of primary sensory neurons in the enteric nervous system (Kirchgessner *et al*, 1992; Furness *et al*, 1995a; Kunze *et al*, 1995). This cannot be considered a totally surprising finding because the gut displays reflex activity when isolated from

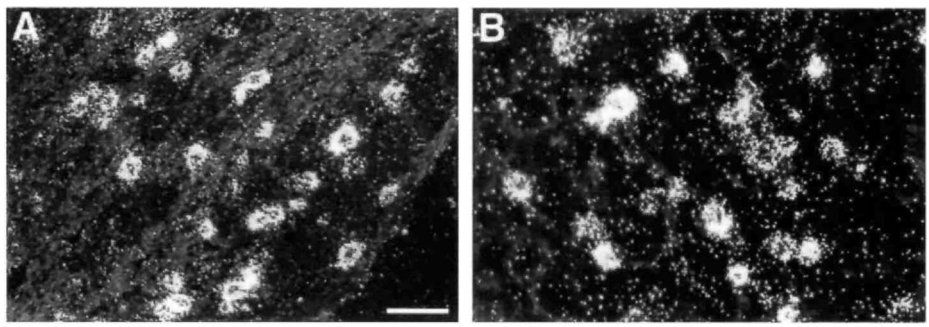

Figure 4. α-CGRP mRNA and SP/NKA-encoding mRNA in primary sensory neurons. Darkfield photomicrographs of autoradiograms of dorsal root ganglia. mRNAs are visualized by silver grains over cell bodies. Aldehyde-fixed cryostat sections of dorsal root ganglia (thoracic level) were processed for *in situ* hybridization histochemistry using [^{35}S]-α-CGRP and β-preprotachykinin anti-sense RNA probes (4 ng/slide and 1 ng/slide, respectively) obtained by *in vitro* transcription and autoradiography as previously described (Sternini *et al*, 1989; Sternini and Anderson, 1992). β-preprotachykinin RNA encodes for both SP and NKA. Autoradiograms were exposed 15 and 10 d, respectively. Clusters of silver grains over individual neurons indicate specific hybridization. Note that α-CGRP and SP/NKA-encoding mRNAs are expressed in numerous sensory neurons of the dorsal root ganglia with an overlapping distribution. *Scale bar*, 150 μm.

the CNS and because the peristaltic reflex, consisting of an oral contraction and an anal relaxation of the muscle following mucosal compression or distension, is evoked *in vitro* (Bayliss and Starling, 1899; Trendelenburg, 1917). Indeed, for this reflex to occur, the enteric nervous system must have primary sensory neurons, sensory receptors, interneurons, and motor neurons. The identification of primary sensory neurons in the enteric nervous system, however, has remained elusive for a long time, and direct evidence for their existence has been provided only during the past few years. The identity of enteric, primary sensory neurons for intrinsic reflexes, however, is still uncertain (Furness *et al*, 1995a; Kunze *et al*, 1995).

Diversity and Versatility of Thin Axon Sensory Neurons and Functional Correlates Thin axon sensory neurons constitute a remarkably diversified class of neurons characterized by several features. They are associated with several classes of transducers (sensory organs), and they sensitize to nociception. They display selective neurotoxin susceptibility to capsaicin, which at low doses exerts a powerful excitatory effect on unmyelinated axons, whereas at high doses administered systemically it can result in the elimination of a large proportion of small ganglion cells and unmyelinated fibers (Kruger, 1987; Holzer, 1988; Kruger, 1988). They display a variety of biochemical features that distinguish them from the large myelinated components. The current concept is that somatic and visceral afferents, in addition to conveying information of potentially noxious stimuli to the CNS, are modulatory at both central and peripheral terminals, they have peripheral neuroeffector functions on appropriate targets, and they undergo activity- dependent long-term changes. The current expanded view of afferent neurons, including visceral afferents, results from a better understanding of the neurochemistry and visceral innervation pathways of afferent neurons. The development of methods for detecting specific chemical markers in sensory neurons has played a major role in our understanding of sensory neuron functions by providing important information on their targets. This, together with information on transmitter release from peripheral terminals and the evidence for receptors for these chemical substances on sensory neuron targets, has provided the morphologic background for a local effector function for sensory nerves. Overall, these morphologic observations have been substantiated by functional evidence, from the antidromic vasodilatation phenomenon to the plasticity of afferents in response to injury and inflammation (Holzer, 1988).

Sensory markers that have been proven useful for the identification of subpopulations of small diameter sensory neurons include neuropeptides, amino acids, purines, nitric oxide, cytoplasmic enzymes, e.g., specific acid phosphatase isoenzymes, and cell surface complex oligosaccharides that can be visualized by specific monoclonal antibodies and, more conveniently, by plant lectins (Kruger, 1987, 1988, 1993). A large proportion of afferents express peptides (Colin and Kruger, 1986; Gibbins *et al*, 1987; Ju *et al*, 1987; Kruger, 1987; Kruger *et al*, 1989). Peptides that have been associated with sensory neurons include bombesin/gastrin-releasing peptide, CGRP, cholecystokinin, dynorphin, galanin, somatostatin, tachykinins, and VIP. CGRP has been one of the most useful peptide marker for the identification of a major subset of visceral afferents and of afferents in general. Indeed, immunolabeling for this peptide has revealed the largest population of small-to-medium size sensory neurons **(Fig 3*A*)** and of peptidergic thin sensory axons distributed to a large variety of targets, including blood vessels and tissue that could be excited by a variety of physical stimuli known to elicit a sensation of pain, including the tympanic membrane, the dental pulp, and the tunica vasculosa of the testis (Colin and Kruger, 1986; Kruger, 1987). CGRP immunohistochemistry also labeled a subpopulation of large sensory neurons, which might be related to mechanoception or other sensory function (Matsuyama *et al*, 1986; Noguchi *et al*, 1990). A large proportion of small-to-medium size sensory neurons expressing CGRP contains SP **(Fig 3*A,B*)** (Gibbins *et al*, 1987; Ju *et al*, 1987). SP has been considered a candidate in primary sensory neurons for the central transmission of afferent information and as a peripheral mediator of neurogenic inflammation (Holzer, 1988). CGRP immunoreactivity represents the expression of distinct genes, the calcitonin/α-CGRP, and the β-CGRP genes (Rosenfeld *et al*, 1983; Amara *et al*, 1985), which are both expressed in sensory neurons with an overlapping pattern; α-CGRP is the predominant form **(Fig 4*A*)**, and it is the form preferentially expressed in large size primary sensory neurons (Noguchi *et al*, 1990; Sternini and Anderson, 1992). Whether these different neurons have distinct projections is unknown. SP also belongs to a family of structurally related peptides that in mammals include NKA and its N-terminally extended forms, neuropeptide κ and γ, which together with SP derive from the preprotachykinin gene I, and the neurokinin B, which is encoded by the preprotachykinin gene II. Hybridization studies indicate that afferent neurons express the products of the preprotachykinin gene I (Sternini *et al*, 1989) **(Fig 4*B*)**. Therefore, sensory neurons immunoreactive for CGRP and SP might contain up to six distinct peptides (i.e., α-CGRP, β-CGRP, SP, NKA, neuropeptide κ and γ), which are encoded by three distinct genes. By combining immunohistochemistry with axonal transport approaches and neurotoxin treatment with capsaicin, it has been possible to identify a variety of targets for these sensory neurons (De Groat, 1986; Dockray and Sharkey, 1986; Kruger, 1988; O'Brien *et al*, 1989; Sternini, 1991; Sternini and Anderson, 1992). These include blood vessels, mast cells, epithelia, glands, lymphoid tissue, dental pulp, and a variety of tissues and cells in the viscera, including secretory cells, neurons, and smooth muscle. Different combinations of peptides can be found in specific groups of primary sensory neurons, and distinct

populations of neurons differing in their neurochemical properties and projection patterns have been identified (Gibbins *et al*, 1987; Ju *et al*, 1987). The multiplicity of peptide expression by a single neuron or by individual populations of neurons might be indicative of different targets and different functions. For instance, sensory neurons projecting to the pelvic viscera contain a different combination of peptides than those supplying the thoracic or the abdominal viscera (De Groat, 1986; Dockray and Sharkey, 1986; Sternini, 1992). Furthermore, an individual peptide may co-localize with different peptides in distinct neuronal classes. The multiplicity of peptides and the variety of peripheral termination sites of peptidergic afferents do not appear to correlate with sensory or reflex function and cannot be reconciled with a pure sensory transmission role. For instance, it is not obvious why the pulp and dentinal tubules of teeth need such a rich supply of CGRP axons because dense innervation is not required to detect injury. On the other hand, the distribution of targets appears more reconcilable with effector roles of these thin sensory fibers. This is further supported by the finding of receptors for sensory peptides in areas that receive peptidergic afferent innervation. For instance, CGRP and SP receptor binding sites and SP receptor immunoreactivity are quite abundant in some of the CGRP/SP afferent targets (Mantyh *et al*, 1989; Sternini *et al*, 1993; Sternini *et al*, 1995b). Furthermore, the observation that peptides are released from peripheral endings of sensory nerves and act upon receptors on cells that are not synaptically contacted by axons provides additional evidence for effector roles of afferents (Kruger, 1987; Holzer, 1988; Kruger, 1988).

The complex organization, the diversity of targets of innervation (from bone and teeth, to sphincters, ducts, glands, chemosensory epithelia, and the iris), the multiplicity of chemical messengers in the same afferents, and the distribution of receptor binding sites and receptors in specific structures that receive afferent innervation are not explicable solely as a function for sensory information. They preclude simple generalizations regarding their putative functional roles and are consistent with a broad functional role by releasing substances such as peptides from their endings in peripheral tissues. Effector roles of peripheral endings of afferent neurons could include control of blood flow and vascular permeability, maintenance of mineralized tissues, regulation of gene expression, and control of autonomic functions. Effector roles are also supported by the plasticity of spinal neural circuits and the ability of peripheral inflammation and hyperalgesia to induce peptide expression in dorsal root ganglia. Plasticity in primary afferent neurons is supported by the alteration of phenotypic expression of primary afferent neurons induced by nerve injury and by the evidence for regulation of gene expression following peripheral inflammation and hyperalgesia or nerve injury (Holzer, 1988; Kruger, 1988; Aberdeen *et al*, 1990, 1992; De Groat and Kruse, 1993; Kruger, 1993). Thus, sensory peptidergic axons might have a daily functional role in addition to the rare event of signaling injury to the CNS (Kruger, 1987, 1988, 1993).

CONCLUDING REMARKS

An emerging principle is the presence of a large number of transmitters/modulators, including peptides, in autonomic and sensory neurons, which often co-exist in individual neurons and are presumably co-released. Phenotypic expression of multiple transmitters and modulators enables neurons to control various functions such as circulation, immune response associated with damage and/or infection, and mobilization of metabolic events underlying inflammatory rejection. For instance, co-transmission might be a mechanism by which autonomic neurons operate in an efficient and controlled manner in response to numerous and various demands. Similarly, in the sensory nervous system, the multiplicity of chemical messengers and their relationship with targets, as revealed by the patterns of distribution of afferent terminals and of peptide receptors, account for multiple effector roles in addition to sensory transmission, which is likely to be a minor role of most afferents, particularly of those supplying deep structures. Finally, an observation worthy of mention is the interaction between autonomic and sensory structures, as evidenced by the rich sensory innervation of sympathetic post-ganglionic neurons and its enhancement following sympathectomy (Matthews *et al*, 1987; Silverman and Kruger, 1989; Aberdeen *et al*, 1990, 1992), which may account for a peripheral mechanism of some forms of hyperalgesia (Levine *et al*, 1986).

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